or 22-36" has been replaced with "SEQ ID NO: 43, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 69, or a complementary sequence of any of such nucleotides." Independent claim 64 has been amended as follows. "[A] biologically active β-secretase protein of any of claims 1-11 or 22-36 or a complementary sequence of any of such nucleotides" has been replaced with "SEQ ID NO: 43, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 69, or a complementary sequence of any of such nucleotides." Support for the amendment to claims 48, 62, and 64 is found in Figures 1A and 2A, and throughout the specification, *e.g.*, support for SEQ ID NO: 43 is found in Figure 2B and at page 7, lines 5-7 of the specification; support for SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 69 is found in Figure 2A, and at page 8, line 4, line 5, and line 7, respectively, of the specification.

The suggested amendments to claims 51 and 66 have been made. Claim 51 has been amended so that it no longer depends from canceled claims 49 and 50. Claim 58 has been amended to recite the β-secretase protein of any one of withdrawn claims 1-10 or 22-35. Claim 60 has been amended to identify P10-P4'staD->V by its SEQ ID NO. Claims 62 and 63 have been amended to depend from claim 61. Claim 62 has been further amended to recite the β-secretase protein of any one of withdrawn claims 1-11 or 22-36.

Claim 68 has been amended to recite the full names of MBP-C125wt and MBP-C125sw. The paragraph beginning at page 2, line 13 of the specification has been amend to recite the full name of MBP-C125sw, and the paragraph beginning at line 21 of page 63 of the specification has been amended to recite the full name of MBP-C125wt. Support for these amendments is found at page 63, lines 9-27. The paragraph beginning at line 21 of page 63 of the specification has been amended to recite the sequence identifiers for MBP-C125wt cleavage site and the MBP-C125sw cleavage site, SEQ ID NO: 54 and SEQ ID NO: 51, respectively.

The Cross Reference To Related Applications section has been replaced with a replacement section, which provides the current status of the priority applications. The suggested amendment to the paragraph beginning at page 9, line 22 has been made.

No amendment should be construed as an acquiescence in any ground of rejection. The paragraph numbering of the office action is used in responding to the office action's comments.

1. *Objections*

1.1 Specification

The Office Action has objected to the specification because it lacks the full names of the β-secretase substrates MBP-C125wt and MBP-C125sw. Amendments to the specification moot this objection. At the first occurrence of the MBP-C125wt, the specification has been amended to recite the full name of MBP-C125wt: a maltose binding protein fused at the carboxy-terminus to the 125 carboxyl-terminal amino acids of APP having the cleavage site of SEQ ID NO: 54. At the first occurrence of the MBP-C125sw, the specification has been amended to recite the full name of MBP-C125sw: a maltose binding protein fused at the carboxy-terminus to the 125 carboxyl-terminal amino acids of APP having the cleavage site of SEQ ID NO: 51.

The suggested amendment to the paragraph beginning at page 9, line 22 has been made.

1.2 Drawings

Formal drawings will be provided before a Notice of Allowance is mailed for the instant application.

1.3 Claims

The suggested amendments have been made in claims 51 and 66.

2. *Rejections*

2.1 35 U.S.C. § 112, second paragraph

Claims 62 and 63 were rejected for lack of sufficient antecedent basis for "antibody." Claims 62 and 63 have been amended to depend from claim 61 to moot the rejection.

Claim 68 was rejected as being indefinite because both claim 68 and the specification allegedly fail to provide explanations of the abbreviations MBP-C125wt and MBP-C125sw. The Examiner's attention is drawn to page 63, lines 9-27 of the specification,

which discusses the construction of MBP-C125wt and MBP-C125sw, both fusion proteins used as β-secretase substrates in the MBP-C125 assay. Figure 19 schematically shows cleavage of the intact MBP-C125wt and MBP-C125sw fusion proteins. The MBP-C125wt and MBP-C125sw cleavage sites are discussed on page 63, lines 21-23 of the specification. The specification and claim 68 have been amended to recite the full names of MBP-C125wt and MBP-C125sw.

2.2 35 U.S.C. § 112, second paragraph

Independent claim 48, and claims 51-57 depending therefrom have been rejected because the specification allegedly "does not reasonable provide enablement for any β-secretase from any biological source as was as man-made." This rejection is respectfully traversed.

As discussed above, amended independent claim 48 is directed to a sequence of nucleotides encoding "SEQ ID NO: 43, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 69, or a complementary sequence of any of such nucleotides." The Office Action acknowledges the specification is enabled for β-secretases having the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 43, SEQ ID NO: 58, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 74, SEQ ID NO: 75.

2.2 [*sic*] Rejection under 35 U.S.C. § 102(b)

Independent claim 48, and claims 51-57 depending therefrom have been rejected under 102(b) as allegedly being anticipated by Powell *et al.* (EP 0 855 444 A2). This rejection is respectfully traversed.

Anticipation under § 102 requires that "each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of Calif.*, 814 F.2d 628, 631 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The "exclusion of a claimed element from a prior art reference is enough to negate anticipation by that reference." *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1574, 224 USPQ 409, 411 (Fed. Cir. 1984). See also MPEP 2131.

Powell *et al.* exclude at least one element that is set forth in Applicants' claims 48 and 51-57. Claims 48 and 51-57 are directed to nucleotide sequences which have a thymine at residue 389 and encode a protein having a valine at residue 130. Powell *et al.* do not disclose such a nucleotide sequence.

It is the Office Action's position the nucleotide sequence (SEQ ID NO: 1) and the amino acid sequence (SEQ ID NO: 2) disclosed by Powell *et al.* are identical to SEQ ID NO: 1 and SEQ ID NO: 2, respectively, of the instant application. Applicants respectfully point out that the SEQ ID NO: 1 disclosed in the instant application differs from SEQ ID NO: 1 disclosed by Powell *et al.* at nucleotide 389. The instant application discloses a thymine residue at position 389, while Powel *et al.* disclose an adenine residue at position 389. (*See* Exhibit 1, attached hereto.) Further, Applicants respectfully point out that the SEQ ID NO: 2 disclosed in the instant application differs from SEQ ID NO: 2 disclosed by Powell *et al.* at amino acid 130. The instant application discloses a valine residue at position 130, while Powel *et al.* disclose an glutamic acid residue at position 130. (*See* Exhibit 2, attached hereto.) Applicants note that neither the query sequence or the database sequence used to prepare the sequence alignment attached to the Office Action is identical to SEQ ID NO: 2 disclosed by Powell *et al.*

The failure of Powell *et al.* to teach SEQ ID NO: 1 and SEQ ID NO: 2 of the present application precludes an anticipation rejection based on this reference. Therefore, the rejection should be withdrawn.

2.3 Rejection under 35 U.S.C. § 103(a)

Claims 58-59 and 61-63 are rejected as allegedly being unpatentable over Powell *et al.* (EP 0855 444) and further in view of Harakas. The rejection is respectfully traversed.

As discussed above, Powell *et al.* do not teach SEQ ID NO: 1 and SEQ ID NO: 2 of the present application. As acknowledged in the Office Action, Powell *et al.* do not teach recovering β-secretase from a culture of host cells capable of producing β-secretase. The

Office Action states "Harakas teaches that affinity matrices may contain as a biospecific ligands enzyme inhibitors or antibodies [*sic*]."

The citation of Powell *et al.* or Powell *et al.* further in view of Harakas does not establish a *prima facie* case of obviousness. Obviousness requires either that the "references must expressly or impliedly suggest the claimed combination or the Examiner must present a convincing line of reasoning as to why the invention would have been obvious in light of the teachings of the references." *Ex Parte Clapp*, 227 USPQ 972, 973 (BPAI 1985). The Examiner must consider "all of the facts." *In re Lunsford*, 148 USPQ 721, 725 (CCPA 1966). The Examiner is not free to "pick and choose" prior art that supports his position. *Akzo v. US International Trade Commission*, 1 USPQ2d at 1241, 1246 (Fed. Cir. 1986). Obviousness is not established where the prior art as a whole "gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful." *In re O'Farrell*, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988).

The Powell *et al.* reference does not expressly or impliedly suggest the claimed invention. Powell *et al.* do not teach recovering β-secretase from a culture of host cells capable of producing β-secretase. The Harakas reference does not expressly or impliedly suggest the claimed invention. Harakas contains no discussion whatever of separating β-secretase from an extract or culture media containing using an affinity matrix. Harakas similarly fails to disclose or suggest use of a β-secretase inhibitor, or an antibody characterized by its ability to bind β-secretase as recited in the present claims.

The motivation asserted by the Examiner for combining the references would have been insufficient to have led one to use an affinity matrix for separation of β-secretase from cell extract or cultured medium, when an affinity matrix is considered as but one choice from a vast repertoire of potential purification procedures. It is well known that the purification of a protein is not an exact science and that any strategy has potential advantages and disadvantages, the full significance of which are unpredictable without empirical experimentation. The advantage of affinity chromatography identified by Harakas would not have appeared to have any particular relevance to separation of β-secretase from cell extract or cultured medium, and would not have motivated the selection of affinity chromatography from

the vast repertoire of available purification methods available. Even assuming *arguendo* that one were motivated to combine the teachings of the cited references, their combination would not have provided a method of separating β-secretase from cell extract or cultured medium, as claimed.

Perhaps recognizing the deficiencies of Powell *et al.* and Harakas, the Examiner attempts to supplement its teaching by imputing additional information to one of ordinary skill that would "further modify the β-secretases" disclosed by Powell *et al.* "by using for purification an affinity matrix method, when the biospecific ligand is a Powell *et al.* inhibitor or antibody. This line of reasoning is unconvincing. The Examiner's position fails to comprehend, *inter alia*, the almost infinite variety of "choices" potentially available, and the failure of the art to provide any guidance for selecting among these choices other than by empirical experimentation. There is, of course, an entire literature of laboratory manuals, textbooks and journal articles devoted to purification of proteins, of which the cited Harakas reference forms a minute part. A brief glance at this literature would have revealed a vast repertoire of potential purification procedures such as precipitation, anion-exchange chromatography, gel filtration, chromatography on hydroxyapatite columns, hydrophobic chromatography, chromatography on immobilized reactive dyes, affinity chromatography, chromatofocusing, and high-performance liquid chromatography. Each of these procedures in turn has numerous variations. From this vast repertoire of potential techniques, the Examiner has failed to identify any reason that one would have selected an affinity matrix for separation of β-secretase from cell extract or cultured medium.

For all of the above reasons, it is respectfully submitted that the Examiner's rejection is erroneous and should be reversed.

2.4 Non-statutory double patenting

Claim 50 has been canceled rendering the provisional rejection moot.

2.5 Statutory double patenting

Claims 48, 51-62 and 64-59 are provisionally rejected under obviousness-type double patenting as allegedly being unpatentable over claims 56 and 61-77 of copending application 09/501,708.

If, upon allowance, the claims of the 09/501,708 application are in conflict with the presently claimed invention, Applicants will address the provisional rejection of claims 48, 51-62 and 64-59 under non-statutory obviousness-type double patenting.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,

Rosemarie L. Celli

Rosemarie L. Celli
Reg. No. 42,397

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (650) 326-2400
Fax: (650) 326-2422
RLC
PA 3215247 v3

b

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Please replace the CROSS REFERENCE TO RELATED APPLICATIONS section with the following replacement section.

Cross-Reference to Related Applications

This application is a nonprovisional[claims the benefit] of U.S. [Provisional]Application No.[Numbers] 60/114,408, filed 12/31/1998, now abandoned, U.S. Application No. 60/119,571 filed 2/10/1999, now abandoned, U.S. Application No. 60/139,172 filed 6/15/99, now abandoned, all of which are hereby incorporated herein by reference in their entireties.

Please amend the paragraph beginning at page 2, line 13 as follows.

This invention is directed to a β-secretase protein and in particular to a purified protein characterized by a specific activity of at least about 1.0×10^5 nM/h/μg protein in a MBP-C125sw substrate assay, which is representative β-secretase assay that uses a maltose binding protein fused at the carboxy-terminus to the 125 C-terminus amino acids of APP having the cleavage site of SEQ ID NO: 51 (hereinafter referred to as "MBP-C125sw").

Please amend the paragraph beginning at page 9, line 22 as follows.

The term "fragment," when referring to β-secretase of the invention, means a polypeptide which has an amino acid sequence which is the same as part of but not all of the amino acid sequence of full-length β-secretase polypeptide. In the context of the present invention, the full length β-secretase is generally identified as SEQ ID NO: 2, the ORF of the full-length nucleotide sequence; however, according to a discovery of the invention, the naturally occurring active form is probably one or more N-terminal truncated versions, such as amino acids 46-501, 22-501, 58-501 or 63-501; other active forms are C-terminal truncated forms ending between about amino acids 450 and 452. The numbering system used throughout is based on the numbering of the sequence SEQ ID NO: 2.

Please amend the paragraph beginning at line 21 of page 63 as follows.

Recombinant proteins were generated with both the 125 C-terminus amino acids of wild-type APP sequence [(MBP-C125 wt)] at the cleavage site (..Val-Lys-Met-Asp-Ala..) (SEQ ID NO: 54) (hereinafter referred to as "MBP-C125wt") or the "Swedish" double mutation [(MBP-C125sw)] (..Val-Asn-Leu-Asp-Ala..) (SEQ ID NO: 51) (also referred to as "MBP-C125sw"). As shown in FIG. 19, cleavage of the intact MBP-fusion protein results in the generation of a truncated amino-terminal fragment, with the new SW-192 Ab-positive epitope uncovered at the carboxy terminus. This amino-terminal fragment can be recognized on Western blots with the same Ab, or, quantitatively, using an anti-MBP capture-biotinylated SW-192 reporter sandwich format, as shown in FIG. 19.

IN THE CLAIMS:

Please amend the claims as follows.

48. (Amended) An isolated nucleic acid, comprising a sequence of nucleotides that encodes SEQ ID NO: 43, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 69,[the β-secretase protein of any of claims 1-10 or 22-35,] or a complementary sequence of any of such nucleotides.

51. (Amended) An expression vector, comprising

the isolated nucleic acid of claim 48[, 49 or 50]; and

operably linked to said nucleic acid, regulatory sequences effective for expression of the nucleic acid in a selected host cell.

58. (Amended) A method of producing a recombinant β-secretase enzyme, comprising culturing a cell transfected with a vector comprising a sequence of nucleotides that encodes SEQ ID NO: 2, SEQ ID NO: 43, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 74, SEQ ID NO: 75, a β-secretase protein, or a complementary sequence of such nucleotides[according to any of claims 53-57]

under conditions to promote growth of said cell, and subjecting an extract or cultured medium from said cell to an affinity matrix.

60. The method of claim 59, wherein said inhibitor molecule is P10-P4'staD->V (SEQ ID NO: 73).

62. (Amended) The method of claim 61[58], wherein said antibody binds specifically to any of the protein compositions of SEQ ID NO: 2, SEQ ID NO: 43, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 74, SEQ ID NO: 75, or a β-secretase protein[claims 1-11 or 22-36].

63. (Amended) The method of claim 61[58], wherein said antibody further lacks significant immunoreactivity with a protein having the sequence SEQ ID NO: 2 [1-501].

64. (Amended) A heterologous cell, comprising

(i) a nucleic acid molecule encoding SEQ ID NO: 43, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 69,[a biologically active β-secretase protein according to any of claims 1-11 or 22-36,] or the complementary sequence of said nucleic acid molecule;

(ii) a nucleic acid molecule encoding a β-secretase substrate molecule; and

(iii) operatively linked to (i) and (ii), a regulatory sequence effective for expression of said nucleic acid molecules in said cell.

66. (Amended) The cell of claim 64, wherein both said nucleic acids encoding said β-secretase protein and encoding said β-secretase substrate molecule are heterologous to said cell.

68. (Amended) The cell of claim 64, wherein said β-secretase substrate is selected from the group consisting of a maltose binding protein fused at the carboxy-terminus to the 125 carboxyl-terminal amino acids of APP having the cleavage site of SEQ ID NO: 54

(MBP-C125wt) and a maltose binding protein fused at the carboxy-terminus to the 125 C-terminus amino acids of APP having the cleavage site of SEQ ID NO: 51 (MBP-C125sw).